GONADS OF GROUPERS (SERRANIDAE) FROM MURUROA AND HAO (FRENCH POLYNESIA) USED AS INDICATORS OF THE QUALITY OF THE CORAL REEF ENVIRONMENT: HISTOLOGICAL AND ULTRASTRUCTURAL ASPECTS

by

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ABSTRACT. - A histological and ultrastructural study was carried out on gonads of 6 species of groupers, from two atolls (Mururoa and Hao in French Polynesia) suspected of being modified by man-made perturbations (radionuclides, ciguatera). Gonadal organization and the fine structure of the germ and somatic cells were apparently unaffected, suggesting that reproductive potentialities are normal.

RÉSUMÉ. - Les gonades de Serranidae de Mururoa et de Hao utilisées comme indicateurs de la qualité de l'environnement des récifs coralliens: aspects histologiques et ultrastructuraux.

Une étude histologique et cytologique a été entreprise sur les gonades de 6 espèces de mérous de deux atolls (Mururoa et Hao, Polynésie française) soupçonnés d'atteintes d'origine anthropique (radionucléides, ciguatera). L'organisation gonadique et les caractères ultrastructuraux des cellules germinales et somatiques ne sont pas modifiés, suggérant que les potentialités génésiques de ces poissons sont demeurées intactes.

Key-words. - Serranidae, *Cephalopholis argus*, *Epinephelus merra*, *E. microdon*, *E. tauvina*, *Plectropomus leopardus*, *Variola louti*, ISE, French Polynesia, Mururoa I., Hao I., Coral reefs, Gonads, Health condition.

Coral reefs have generally been thought to be characterized by their striking biological richness, with a high diversity of fish. However, over the last few decades, reports of coral reef deterioration, both from natural (hurricanes, the effects of predators, etc.) and anthropogenic (pollution, terrigenous sedimentation, etc.) events, have given cause for alarm. The large number of hazards threatening coral reefs, and especially coral reef fish, has been emphasized (McAllister, 1988; Grigg and Dollar, 1990; Roberts, 1993). The possibility of radioactive contamination is one such hazard (Wells and Hanna, 1992).

An assessment of the impact of 41 atmospheric nuclear tests (1966 to 1974) and then 63 underground nuclear tests (1975 to 1991) on the South Pacific environment at Mururoa in French Polynesia has provoked controversy (Ribbe and Tomczak, 1990; Buske, 1991; Doury, 1993). Man-made radioactivity has been measured in the water of Mururoa lagoon, an island situated in the Pacific Ocean (21°50'S, 138°50'W), in the Southern part of the Tuamotu Archipelago. The artificial radioactivity now present in the lagoon (1.4×10^8 m^2) comes from world-wide airborne fallout and from the release of radionuclides from the sediment near the sites of the tests. The level of ^{137}cesium was first

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estimated in 1987 by a scientific mission (Cousteau, 1988, cited by Ribbe and Tomczak, 1990) at 3.1 Bq/m^3, and had decreased to 2.8 Bq/m^3, in 1990 (Bourlat *et al.*, 1992). A mean value of 2.79 Bq/m^3 was recently proposed by Bourlat and Martin (1992). In addition, the level of $^{239-240}$plutonium was low (0.4-0.5 Bq/m^3) (Bourlat *et al.*, 1992, 1995) and no trace of ^{134}cesium was detected (Bourlat and Martin, 1992; Bourlat *et al.*, 1994). However ^{137}cesium has been found to accumulate in groupers (*Epinephelus* sp.) from experimental testing in both Brazil (Guimaraes, 1992) and in another Tuamotu atoll, where they showed a concentration factor of between 84 and 260 (Meyer and Ducousso, 1981).

The dinoflagellate *Gambierdiscus toxicus*, aetiologic agent of the ichthyosarcotoxism called "ciguatera poisoning", is known to be present inside Mururoa lagoon (Ruff, 1989). The incidence of this toxic alga is not generally believed to be linked with any radionuclide pollution, in contrast to the speculations of Helfrich (1960 in Ruff, 1989) and Danielsson (1984). Ciguatera poisoning is known in many other Polynesian reefs (Gambier Islands, Tuamotu Archipelago: Bagnis, 1969). Ciguatera outbreaks also have been described in the Hao atoll by Bagnis (1969), Bagnis *et al.* (1985) and Ruff (1989).

Thus an evaluation of any impact of nuclear experiments in the field, on the fish from the reef ecosystem at the Mururoa site itself, is required. A scientific mission was undertaken, in order to collect samples of herbivorous and carnivorous fish from the different sites in the lagoon. The target organs, gonad and liver, were collected and examined cytologically to detect possible pathological abnormalities which might result from an impairment of their environment (Yevich and Barszcz, 1983). Detrimental effects on fish populations through visceral lesions (review: Bruslé, 1993) have been found most frequently in areas in which anthropogenic degradation of the water quality was suspected, as observed in the USA (Grizzle, 1990) and the North Sea (Watermann and Kranz, 1992).

Among the different target organs used in assessing a fish's health are the gonads, the cellular quality of which provides an accurate evaluation of the reproductive potential of the fish. In addition, owing to the high sensitivity of germ cells to radioactivity (Shimada and Egami, 1982), pathological features found in fish gonads might be expected to constitute good indicators of radioactivity effects and consequently provide information on the degree of impairment of the quality of the environment (Bruslé and Bruslé-Sicard, 1992; Bruslé, 1993).

The present work deals with the detection of possible anomalies in the gonads of groupers in order to compare fishes from the Mururoa lagoon (impact of both radionuclides and ciguatera toxin) with those from the Hao lagoon (ciguatoxin alone). Groupers are long-lived, carnivorous fish which constitute convenient targets for checking the effects of both radionuclides (because of their ability to concentrate ^{137}cesium: Meyer and Ducousso, 1981; Guimaraes, 1992) and ciguatera toxins (because of their place high in the food chain: Randall, 1980; Withers, 1988).

MATERIALS AND METHODS

During a scientific mission (from November to December 1990), 48 Serranidae: 23 *Cephalopholis argus*, 9 *Epinephelus microdon* and 6 *Plectropomus leopardus*, together with other groupers such as 5 *E. tauvina*, 3 *E. merra* and 2 *Variola louti*, were caught by spear-fishing, at depths ranging from 3 to 15 m, from 33 stations at Mururoa and 4 stations at Hao in French Polynesia.

Gonads were collected from live fish on board ship immediately after they were caught. Portions of the gonads for histological investigations were fixed in Bouin-Holland's fluid at the time of dissection and then dehydrated and embedded in paraffin. Sections were stained with erythrosin-toluidine blue for light microscopy. Other pieces were fixed in 3.5% glutaraldehyde buffered to pH 7.3 with 0.13 M cacodylate buffer for 3 h at 4°C, post-fixed in 2% osmium tetroxide for 2 h at 4°C, dehydrated in a graded acetone series and finally embedded in epon. Semi-thin sections were stained with methylene blue-II azur (Richardson *et al.*, 1960) for light microscopy and, for TEM, ultra-thin sections contrasted with uranyl acetate and lead citrate solutions (Reynolds, 1963).

In addition, samples of liver and muscle were collected and deep-frozen in order to test for the presence of ciguatera toxins. These samples were tested for toxicity in the Institut Malardé (Papeete, Tahiti) by Dr A.M. Legrand, using a mosquito test: intrathoracic injection of crude extracts into *Aedes aegypti* and evaluation of LD50 (g.fish/mosquito). This test developed by Chungue *et al.* (1984) is considered as one of the most reliable bioassays presently available (Bruslé, 1997).

RESULTS

General gonad morphology

The gonads of all groupers comprised two hollow sausage-shaped lobes, suspended from the peritoneal wall and joined together posteriorly at the genital pore. They were covered by a thick muscular tunic. Microscopic examination was necessary to establish the sexual status of each sample.

Using light microscopy, three types of gonad could be recognized: ovaries, testes and transitional.

In ovaries, the ovarian lamellae projected into the gonad cavity and enclosed female germ cells, especially oogonia and previtellogenic oocytes (at the primary growth phase) in immature or resting ovaries (Fig. 1) and oocytes at different stages of vitellogenesis in the maturation period (Fig. 2).

In testes, the seminiferous lobules contained spermatocysts enclosing the male germ cells (spermatogonia, primary and secondary spermatocytes, spermatids, spermatozoa). All germ cells in a cyst were at the same stage of development (Figs 3, 4). All male groupers sampled showed active spermatogenesis.

Two types of transitional gonads were recognized. The first displayed an ovarian organization, but spermatocysts enclosing differentiating male germ cells (spermatogonia, primary and secondary spermatocytes, spermatids) were scattered among previtellogenic oocytes, within the ovarian lamellae (Fig. 5). The second type had a recognizable testicular organization, with seminiferous lobules and some previtellogenic oocytes in lamellae suspended in the ex-ovarian cavity (Fig. 6).

Transitional gonads were relatively scarce (4 out of 23 in *Cephalopholis argus*, 2 out of 6 in *Plectropomus leopardus*), and reflect two stages in the protogynous sex inversion of groupers, characterized by the settlement of male germ cells within ovarian lamellae concurrent with regression of female germ cells.

The gonads of male, female, and sex inverting groupers did not exhibit any gross structural defects. A study using the transmission electron microscope was carried out on *Cephalopholis argus* in order to evaluate possible cytological damage.

Cytological features of germ cells

The main cellular types recognized in the ovaries were oogonia (11.8 ± 1.1 µm long × 8.5 ± 1.0 µm wide), oval or round in shape, characterized by regular outlines and a low electron-density (Fig. 7). Oogonia, surrounded by follicle cells, exhibited a dense fibrillar material, called "ciment" when it is associated with mitochondria and "nuage" when it is independent of them (Fig. 7). Oocytes at different stages of previtellogenesis in resting ovaries were recognized by their large size (up to 80-100 µm in diameter) and their cytoplasm richly endowed with ribosomes. Nuclei were large (up to 5-6 µm) and numerous nucleoli were located in the peripheral nucleoplasm of the largest previtellogenic oocytes (Fig. 8). Membrane organelles (endoplasmic reticulum, Golgi complexes, mitochondria) were distributed in the cytoplasm of both oogonia and previtellogenic oocytes (Figs 7, 8).

In the testes, spermatogonia (12.9 ± 1.2 µm long × 8.9 ± 1.1 µm wide), oval in shape (Fig. 9), were surrounded by Sertoli cells. The latter were fairly similar to follicle cells, just as the spermatogonia resemble oogonia (regular outlines, low electron density). Spermatocytes, clustered together within cysts delimited by a single layer of Sertoli cells, were contiguous being connected by cytoplasmic bridges. The primary spermatocytes were easily recognized by their synaptonemal complexes (Fig. 10) while the smaller secondary spermatocytes, in metaphase, could occasionally be identified (Fig. 11). Spermatids of decreasing size at different stages of spermiogenesis were characterized by irregular, dense strands of chromatin (Fig. 12). Spermatozoa lacked acrosome and possessed a round nucleus with dense chromatin granules and a few mitochondria.

Besides the cells described above, another cellular type was identified in male, female and sex-inverting *C. argus*. Infrequent in ovaries, these cells were quite abundant in both transitional gonads and testes. They were characterized by irregular outlines with narrow pseudopodium-like extensions (Fig. 13) and by a high electron-density related to a fine granular chromatin and many free ribosomes (Fig. 14). Oval in shape (9 ± 1.2 µm long × 5.4 ± 1.1 µm wide), they exhibited a voluminous nucleus (nucleus-cell ratio = 0.4 ± 0.12), sparse membrane organelles, ciment and nuage. Thus, these germ cells with a high nucleus to cell ratio, abundance of ribosomes and scarcity of membrane organelles displayed the features of undifferentiated cells. This indicates that they are probably earlier than spermatogonia or oogonia and, for this reason, they are

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Abbreviations used in figures

b.l.	:	basal lamina	ov.c.	:	ovarian cavity
ce.	:	centriole	ov.l.	:	ovarian lamella
ch.	:	chromosome or chromatine	P.G.C.	:	Primordial Germ Cell
ci.	:	"ciment"	p.l.	:	pseudopodium-like
f.	:	flagellum	P.O.	:	Previtellogenic Oocyte
F.C.	:	Follicle Cell.	S.C.	:	Seminiferous Cysts
M.O.	:	Meiotic Oocyte	S.L.	:	Seminiferous Lobules
mi.	:	mitochondria	Sc.	:	Spermatocyte
mt.	:	microtubule	Se.C.	:	Sertoli Cell
n.	:	nucleolus	Sg.	:	Spermatogonium
N.	:	Nucleus	So.C.	:	Somatic Cell
n.e.	:	nuclear envelope	St.	:	Spermatid
nu.	:	"nuage"	Sy.c.	:	Synaptonemal complex
Og.	:	Oogonium	Sz.	:	Spermatozoon
			V.O.	:	Vitellogenic Oocyte

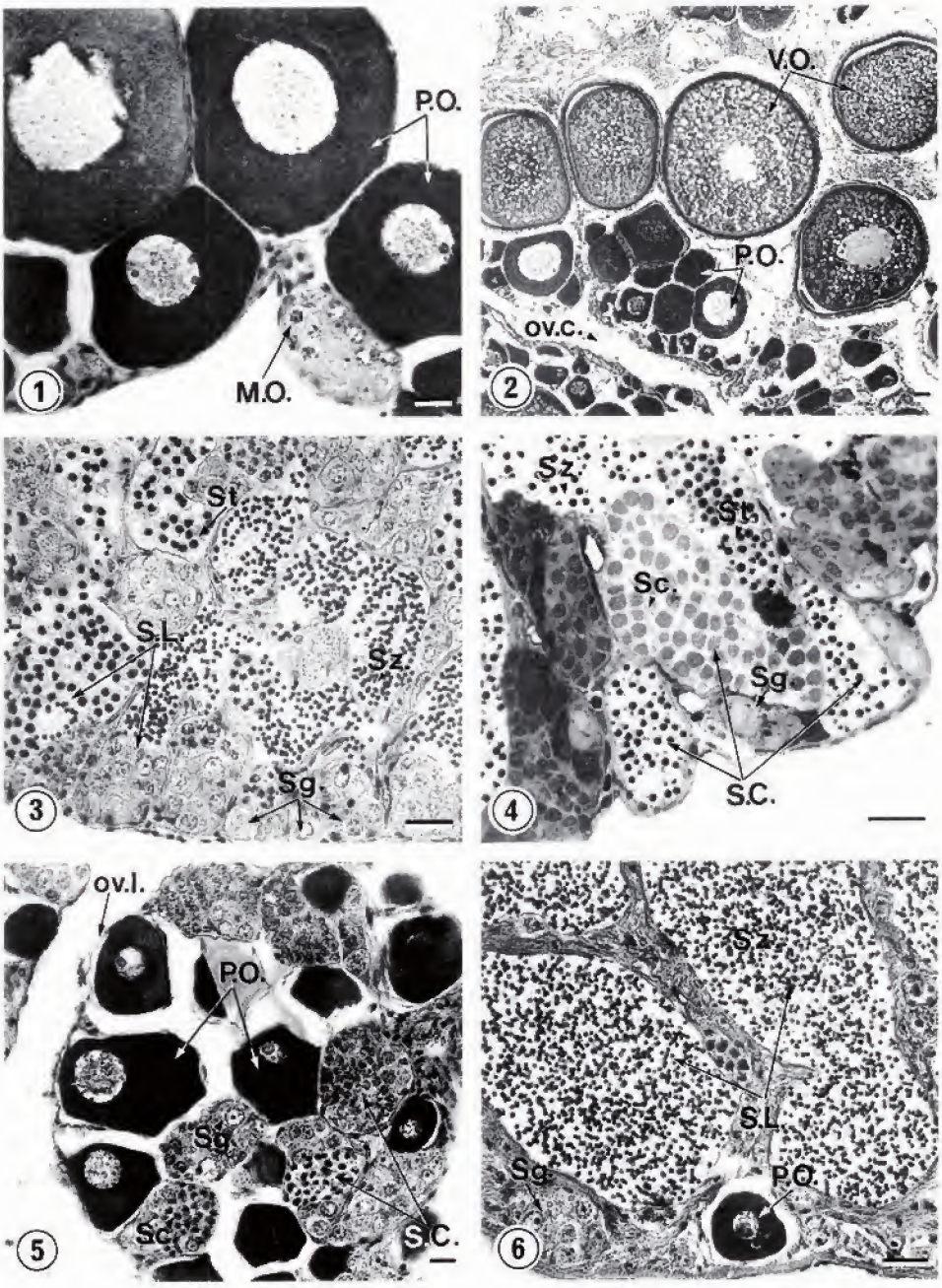
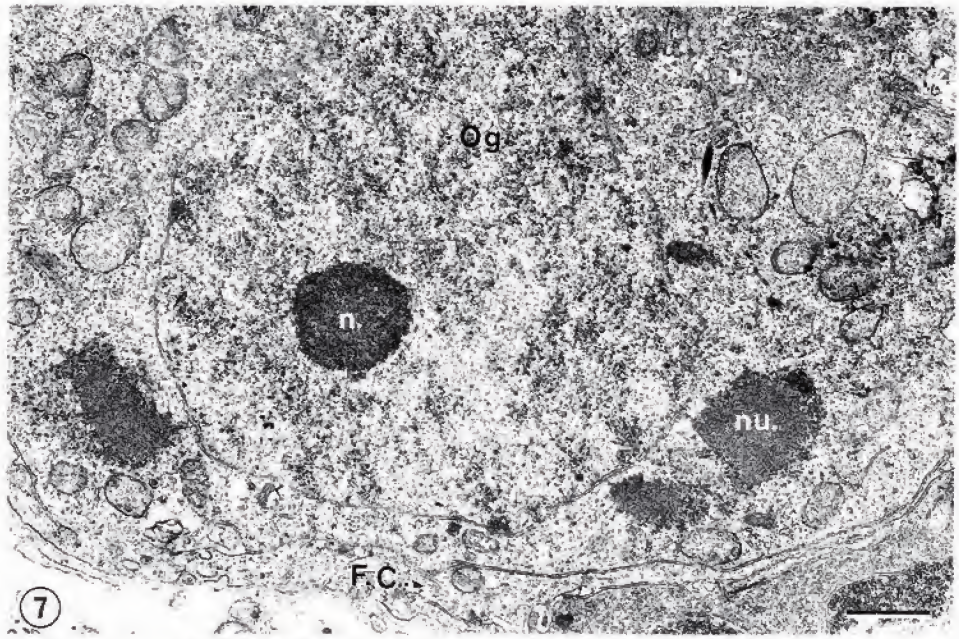


Fig. 1. - Resting ovary; Fig. 2. - Active ovary; Figs 3, 4. - Active testes; Fig. 5. - Transitional gonad: seminiferous cysts scattered within an ovarian lamella. Fig. 6. - Transitional gonad: previtellogenic oocyte between seminiferous lobules. Scale bars = 20 µm.



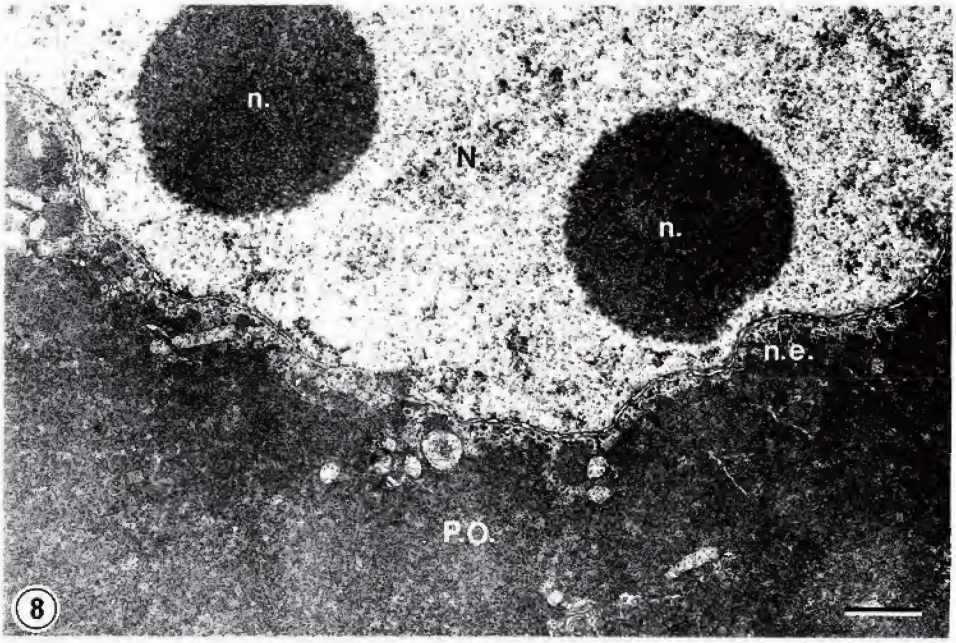
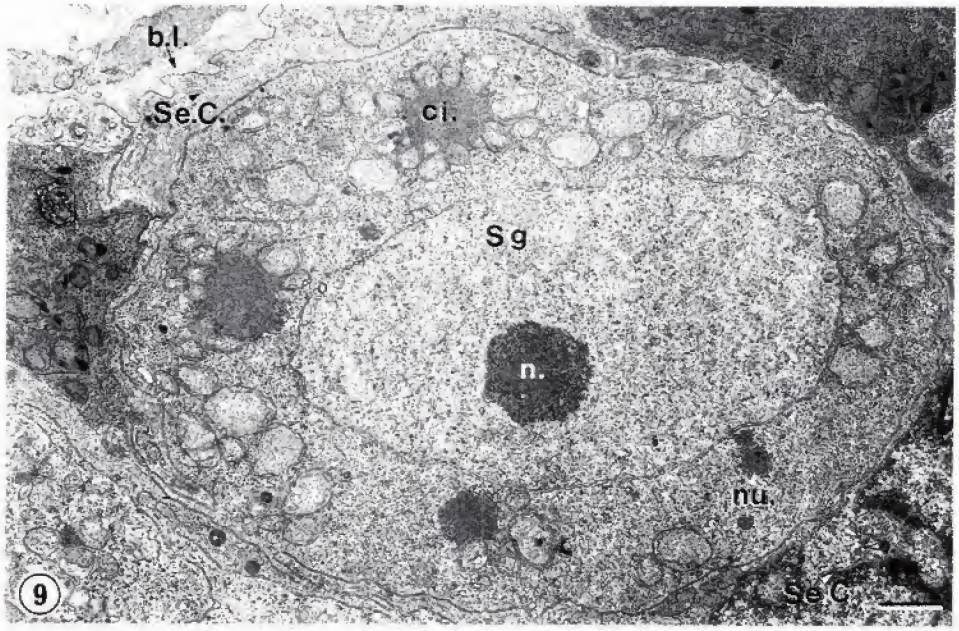


Fig. 7. - Oogonium; Fig. 8. - Previtellogenic oocyte. Scale bars = 1 µm.

called intragonadic primordial germ cells (PGCs). PGCs were surrounded by somatic cells fairly similar to Sertoli and follicle cells.



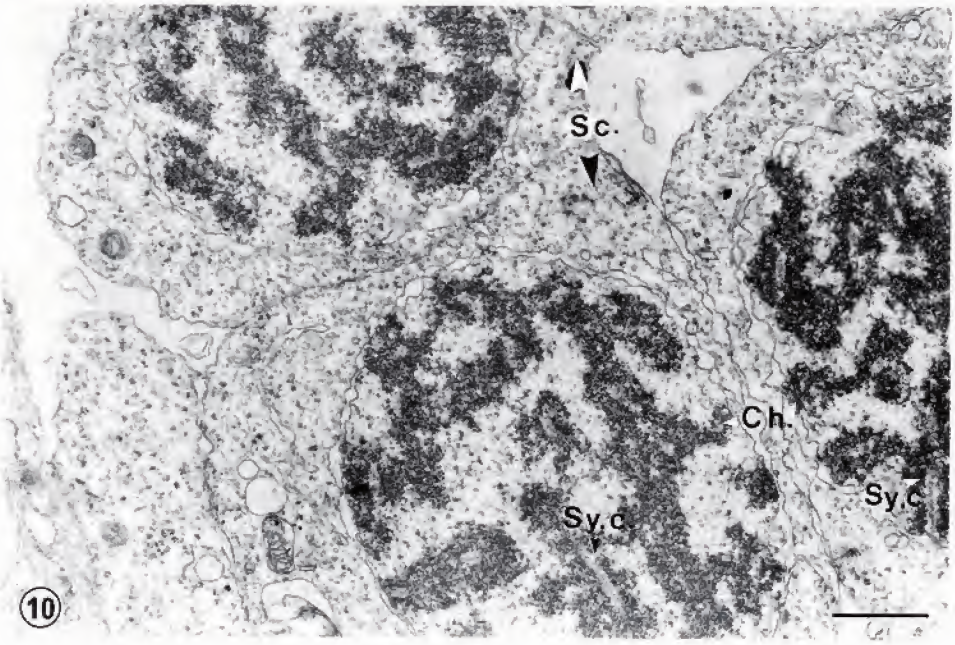


Fig. 9. - Spermatogonium; Fig. 10. - Primary spermatocytes. Scale bars = 1 µm.

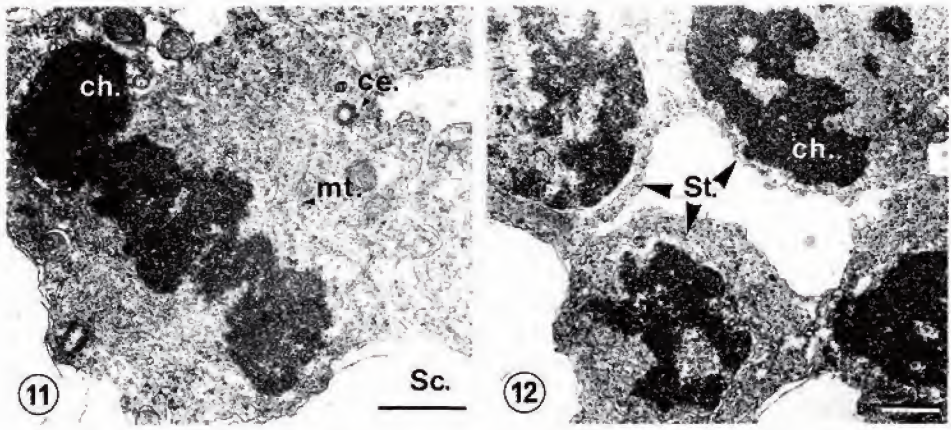


Fig. 11. - Secondary spermatocyte; Fig. 12. - Spermatids. Scale bars = 1 µm.

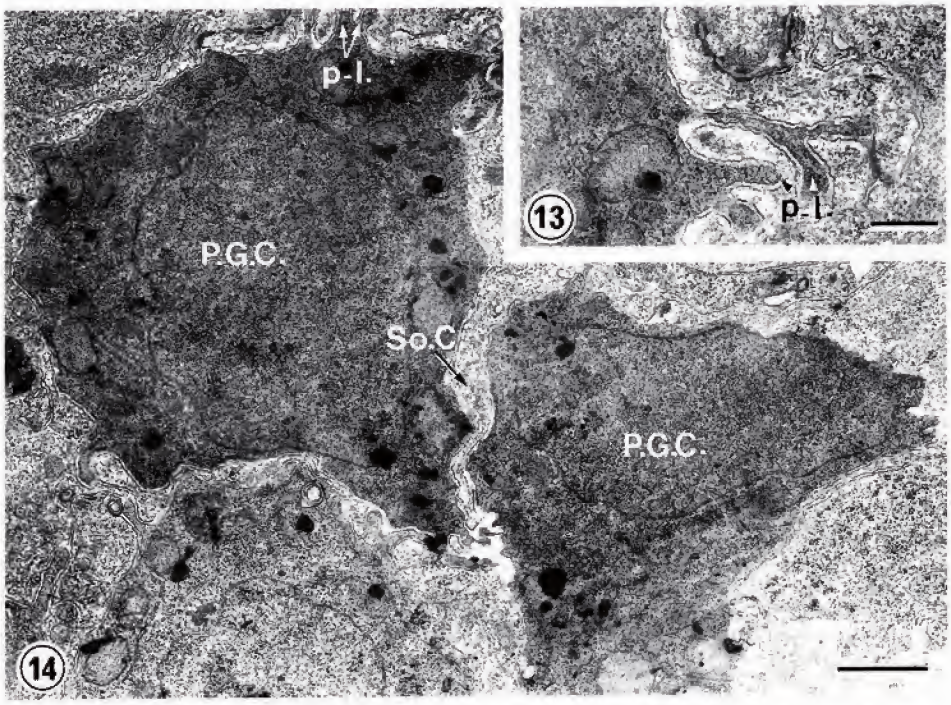


Fig. 13. - A part of a PGC: pseudopodium-like extension. Scale bar = 0.5 µm; Fig. 14. - Primordial germ cell (PGC). Scale bar = 1 µm.

Both the germ and somatic cells described above exhibited a normal fine structure. Neither abnormality nor cellular lesion (pycnosis, vacuolization of organelles, myelinic figures, residual bodies) was found, suggesting the absence of any pathology.

Evaluation of toxicity

For samples from Mururoa, positive (not quantitative) responses were found in 18 samples (72%) and two negative with 6 doubtful responses. For samples from Hao, there were 5 positive (63%), 2 negative and 1 doubtful responses. No association was established between tissue toxicity and the gonadal state.

DISCUSSION

The accumulation of radionuclides, especially ^{137}cesium, has been tested in groupers (*Epinephelus* sp.) through experimental uptake from water (Guimaraes, 1992) or dietary intake (Meyer and Ducousso, 1981). However, these studies made no attempt to evaluate either the radionuclide density inside gonads or any pathological effects of the radionuclides on the gonadal tissues.

Although the gonads are rarely included in most pathological examinations, several authors have drawn attention to the experimental effects of radiation on the gonads of a small number of fish such as the medaka *Oryzias latipes* (Egami *et al.*, 1967; Egami and Hyodo-Taguchi, 1969; Hyodo-Taguchi and Egami, 1976; Michibata, 1976; Konno and Tashiro, 1982; Shimada and Egami, 1982), and the rainbow trout (Zakharova, 1983). These studies indicate that, as in mammals (Mandl, 1964; Baker, 1971), the gonads of fish are very susceptible to experimental damage by X-radiation (Zakharova, 1983) and radiation produced by ^{137}Cs and ^{60}Co (Shimada and Egami, 1982; Konno and Tashiro, 1982). Destructive processes and degenerative changes, even leading to permanent sterility of gonads, can be induced by such irradiation. At the ultrastructural level (Shimada and Egami, 1982), the effects of radiation damage are particularly evident for the membraneous organelles of both undifferentiated and poorly differentiated cells (PGCs and meiotic germ cells, especially spermatocytes): the nuclear envelope and ER were fragmented, mitochondria were swollen and myelin-figures were present in the cytoplasm. In the field, some silver carp *Hypophthalmichthys molitrix* from a nuclear cooling pond having survived the 1986 Chernobyl nuclear power plant disaster, showed gonadal disorders: there were changes in gonadal morphology, with degeneration of germ and somatic cells and even sterility (Belova *et al.*, 1994; Makeyeva *et al.*, 1995). Thus, owing to the high sensitivity of the germ cells in response to various perturbations of intrinsic and extrinsic origin (review *in* Bruslé and Bruslé-Sicard, 1992), gonads can be used as a good indicator of fish health.

In our ultrastructural observations, not only were differentiating male and female germ cells undamaged, but so also were the undifferentiated and poorly differentiated germ cells such as PGCs, oogonia and spermatogonia, suggesting that the germinal potentialities of the groupers were intact. In addition, as the somatic cells surrounding the germ cells were also healthy, the cellular interactions which control gametogenic processes (Fostier *et al.*, 1983) appear to be quite normal. Thus, no overt negative effect of radionuclides can be found in gonads of groupers from the two sites of Mururoa and Hao, of which the former is suspected of being more contaminated than the latter.

Outbreaks of ciguatera poisoning have occured throughout French Polynesia (Bagnis *et al.*, 1979), including Mururoa and Hao Atolls (Bagnis, 1969; Bagnis *et al.*, 1985). This ichthyosarcotoxism is known to develop on reefs where the ecosystem is disturbed, by either natural or man-made perturbations, both of which induce the settlement and growth of toxic benthic dinoflagellates such as *Gambierdiscus toxicus*. A rela-

tionship between radioactive contamination and ciguatera development has been suggested (Helfrich, 1960 *in* Ruff, 1989), although the connection may be indirect (Danielsson, 1984; Ruff, 1989). The gonads of groupers are known to accumulate ciguatera toxins (Bagnis *et al.*, 1979) although this was not tested here. The present finding of toxicity in numerous carcasse samples using the mosquito-test was not associated with any apparent pathology of the gonads. Thus all the germinal populations, especially the early germ cells such as PGCs, oogonia and spermatogonia (probably involved in the protogynous sex inversion in *Cephalopholis argus* as known in other groupers: Shapiro, 1987; Bruslé-Sicard *et al.*, 1992), which are considered to be the most sensitive stage of gametogenesis (see above), displayed normal cellular characters and appeared to be healthy.

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